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T-cell exhaustion: characteristics, causes and conversion

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Summary

T-cell exhaustion is characterized by the stepwise and progressive loss of T-cell functions and can culminate in the physical deletion of the responding cells. Exhaustion is well-defined during chronic lymphocytic choriomeningitis virus infection and commonly develops under conditions of antigen-persistence, which occur following many chronic infections that are of significant public health concern including hepatitis B virus, hepatitis C virus and human immunodeficiency virus infections, as well as during tumour outgrowth. Exhaustion is not a uniformly disabled setting as a gradation of phenotypic and functional defects can manifest, and these cells are distinct from prototypic effector, memory and also anergic T cells. We are gaining insights into the extrinsic and intrinsic factors that determine the severity of exhaustion. These include the duration and magnitude of antigenic activation, availability of CD4 T-cell help, the levels of stimulatory and suppressive cytokines, as well as the expression of activatory and inhibitory receptors. More information is now becoming available regarding the molecular mechanisms that attenuate the responsiveness of exhausted T cells. As the parameters that dictate exhaustion are more thoroughly defined, this is fostering the development of methods that prevent and rejuvenate functionally inferior responses. In this article we discuss our current understanding of the properties of exhausted T cells and the mechanisms that promote and maintain this state.

Keywords: chronic infections; cytokines; effector functions; inhibitory receptors; T cell exhaustion

Qualitative differences in T-cell responses

T-cell exhaustion was first described as the clonal deletion of virus-specific CD8 T cells that occurs during high-grade chronic infections.[1] Technological advancements, especially the production of major histocompatibility complex multimers which can identify antigen-specific T cells without relying on functional readouts, as well as the development of enhanced methods to assess the phenotypic and functional portfolios of single cells have improved our understanding of the complexities of the exhausted state.[2] It is now clear that T cells are not necessarily physically deleted under conditions of antigen persistence but can instead become functionally inept and incapable of elaborating the usual array of effector activities typically associated with robust, protective, effector and memory T-cell populations. Exhaustion is not limited to CD8 T-cell responses as CD4 T cells have also been shown to develop functional unresponsiveness following several infections.[3–11] During the last decade the characteristics of exhausted cells have become better defined and we now appreciate that exhausted T cells are quite distinct from prototypic effector and memory T cells (Fig. 1).

Exhausted T-cell responses have been documented following numerous infections, including lymphocytic choriomeningitis virus (LCMV), polyoma virus, adenovirus, Friend leukaemia virus, mouse hepatitis virus, human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV), and have also been observed in patients with malignancies.[1,6,10,12–29] In each of these cases initial T-cell responses are elicited, but a spectrum of phenotypic and functional defects arises as the responding cells lose their functional capabilities in a progressive and stepwise manner. Interleukin-2 (IL-2) production is one of the first effector activities to be

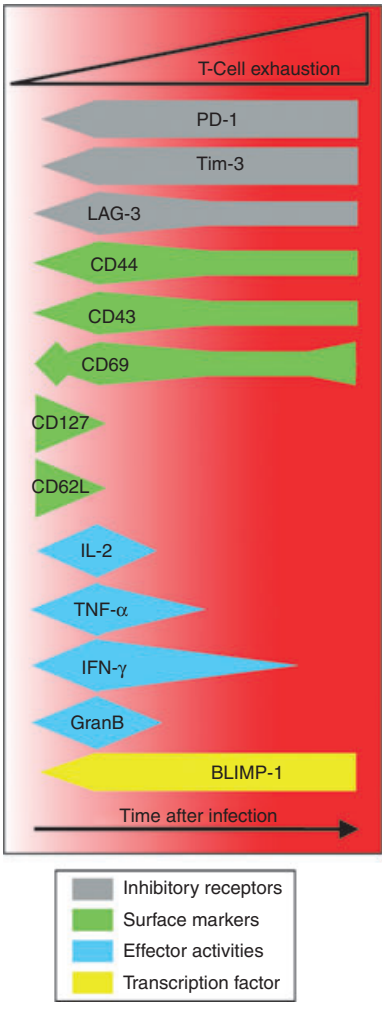

Figure 1. As exhausted T cells emerge sequential phenotypic and functional changes occur. Exhausted T cells express arrays of inhibitory molecules and distinctive patterns of cytokine receptors, transcription factors and effector molecules, which distinguish these cells from conventional effector, memory and anergic T cells. The changes depicted represent only a small subset of the overall alterations that manifest as the exhausted state develops. GranB, granzyme B; IFN-γ, interferon-γ; IL-2, interleukin-2; TNF-α, tumour necrosis factor-α.

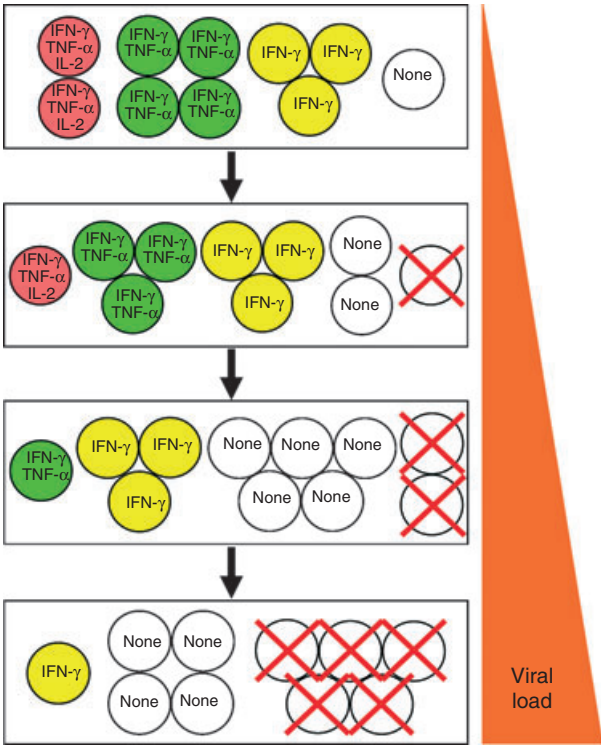

Figure 2. T-cell exhaustion develops in a stepwise and progressive manner and can culminate in the deletion of the virus-specific T cells. The overall ensemble of T cells elicited during the initial stages of infection is comprised of a series of subsets with different functional attributes. If the infection is not brought under control then composition of this population changes as the more polyfunctional cells are lost. Sustained high viral loads result in further reductions in the functional potential of the population and severely exhausted T cells are not maintained. IFN-γ, interferon-γ; IL-2, interleukin-2; TNF-α, tumour necrosis factor-α.

extinguished, followed by tumour necrosis factor-α (TNF-α)production, whereas the ability to produce interferon-γ (IFN-γ) is more resistant to inactivation (Fig. 2) [6,7,15] Although cytotoxic functions by exhausted T cells can be difficult to measure *in vitro*, sensitive *in vivo* assays can detect some level of killing activity.[6,30] The biological significance of this is uncertain because these cells do not contain the infection. Counterintuitively, despite their functional ineptness, exhausted T cells display high levels of CD43 (1B11), CD69 and inhibitory receptors but low levels of CD62L and CD127, which is an expression profile typically associated with effector T cells (Fig. 1).[6,7,15,26,31–34] Unlike prototypic memory T cells that are maintained at remarkably stable levels in the absence of antigen, severely exhausted T cells can succumb to deletion. This physical loss of the cells is probably the result of several factors including shifts in the expression of pro- and anti-apoptotic factors as well as an inability to respond to IL-7 and IL-15, which normally regulate T-cell homeostasis.[22,31,32,35]

Exhausted T cells most commonly emerge during high-grade chronic infections, and the levels and duration of antigenic stimulation are critical determinants of this process.[6,36–38] Infections elicit multi-epitope-specific T-cell responses, but not all specificities are equally prone to exhaustion.[6,15,26,34] This differential silencing of responses, even within the same host, may be consequential because the specificities of T cells that are most effective at eradicating the pathogen may become functionally inactivated more rapidly and quickly deleted. Decreasing antigen availability, as occurs during the gradual resolution of infections and following intervention strategies that promote viral control, generally help the exhausted T-cell population regain polyfunctional attributes and more

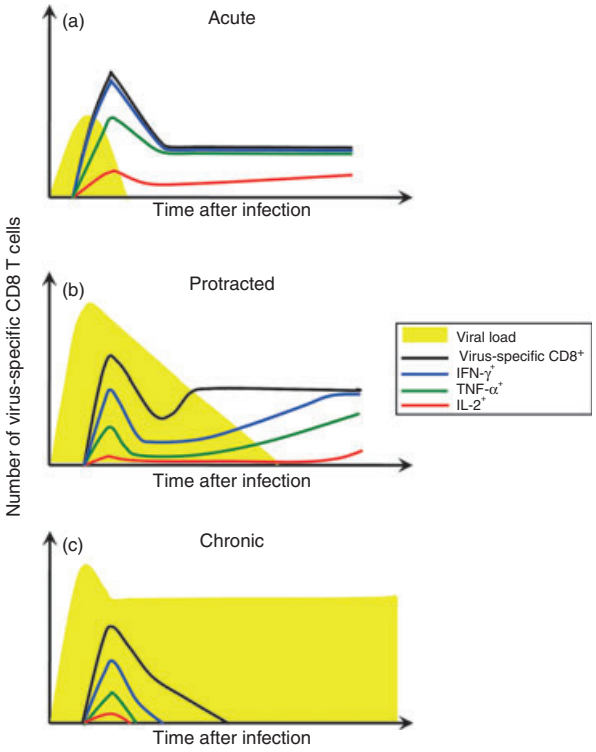

Figure 3. Divergent patterns of CD8 T-cell responses develop following acute, protracted, and chronic infections. (a) Acute viral infections are rapidly contained and establish stably maintained memory T-cell pools. These memory T cells are capable of elaborating many effector functions including interferon-γ (IFN-γ) and tumour necrosis factor-α (TNF-α) production and a subset produce interleukin-2 (IL-2). (b) During more protracted infections the initial burst size and the functional quality of the response are often reduced, and further development of an exhausted phenotype occurs as viral loads remain high. Gradual resolution of the infection may result in a progressive resurgence of the response. (c) Exhaustion develops more rapidly and the responding T cells do not recover function if viral loads are not contained and a high-grade chronic infection ensues. (Based on Fuller *et al.*, [6])

closely match typical memory T cells (Fig. 3).[4,6,31,39–43] As with the development of the effector-function-negative state, the resurrection is also unequal and severely exhausted T cells may be refractory to reactivation.[6,22,44]

In addition to antigen availability other factors, including cytokine levels and the presence of CD4 T cells dictate the development of exhaustion, as discussed further in this article. In many ways this loss of effectiveness is paradoxical because the principle function of the immune response is to protect the host. So why inactivate the response? Perhaps the cardinal rule is to 'do no harm' and the stepwise mechanism of dampening T-cell responses that are overwhelmed by a chronic infection provides the flexibility to allow a level of infection control while attenuating immunopathology.

CD4 T cells help sustain CD8 T-cell responses

CD4 T cells have multiple effects on the overall immune response following infection and are often required for optimal CD8 T-cell responses. CD4 T cells deliver these helper functions in several ways. They activate professional antigen-presenting cells such as dendritic cells via CD40 and CD40 ligand interactions, secrete chemokines and cytokines, which guide naive T cells to the sites of priming in secondary lymphoid organs and activated T cells to the location of the infection, and also produce supportive cytokines including IL-2 and IL-21, which can act directly on the responding CD8 T cells.[45–52] As a consequence, any abnormalities in, or loss of, CD4 T cells during the course of an infection, which can occur as these cells succumb to exhaustion during HBV, HCV, or LCMV infections; or undergo deletion during HIV-1 infection, probably feed back on CD8 T cells, rendering them less effective.[3–11] The absence of CD4 T cells during acute infections can result in the generation of defective memory CD8 T cells, which may exhibit altered homeostatic turnover, a reduced functional repertoire including the loss of IL-2 production, and the inability to mount vigorous secondary responses.[53] Under conditions of antigen-persistence, which occurs during chronic infections, the requirements for CD4 T cells are even more stringent because CD8 T cells generally succumb to severe exhaustion and may be deleted without T-cell help.[26]

Several recent studies have demonstrated a pivotal role for IL-21, probably produced by CD4 T cells, in sustaining CD8 T-cell responses to chronic infections.[49–51] The absence of IL-21 or its receptor does not have a significant impact on primary CD8 T-cell responses induced following infection with LCMV clone 13 or Docile strains; however, the virus-specific cells subsequently lose their ability to produce IFN-γ, TNF-α and IL-2, and also display other hallmarks of the exhausted state including high levels of CD43 (1B11) and programmed death-1 (PD-1) expression. Significantly, IL-21 or IL-21 receptor-deficient mice are unable to control the infection, and these outcomes closely parallel those observed in CD4-deficient mice, implicating IL-21 as a vital helper factor.[49–51] During HIV-1 infection the levels of IL-21 in the circulation correlate with CD4 T-cell counts, and individuals with higher levels of IL-21 have increased frequencies of HIV-specific CD8 T cells.[54] In CD4-deficient mice infected with LCMV clone 13, the administration of IL-21 has been shown to reduce viral loads, enhance the responsiveness of the virus-specific CD8 T cells and suppress the usual deletion of epitope-specific populations which quickly succumb to exhaustion.[51] A downside to the IL-21 therapy is that the treated mice became moribund, most likely as a result of immunopathology caused by the enhanced immune response.

Interleukin-21 has pleiotropic effects so it is likely that it impacts multiple aspects of the immune response in persistently infected hosts. Nevertheless, simultaneous comparisons of the activities and fates of CD8 T cells which express the IL-21 receptor, and can perceive IL-21-dependent signals, with those cells which cannot, show that IL-21 acts directly on the CD8 T cells to sustain their presence and activity during chronic LCMV infections.[49–51] The related and genetically linked common γ-chain cytokine IL-2 has also been shown to augment CD8 T-cell responses, and CD8 T cells which cannot perceive IL-2 because of IL-2 receptor-α chain deficiency are also rapidly lost during chronic LCMV infections.[47,55–57] IL-21 appears to have far less of an impact on the induction and maintenance of anti-viral CD8 T cells following acute infections.[49,50] Given the differential requirements for IL-21 during acute and chronic infections, and that this cytokine has been proposed to limit the rapid generation of terminally differentiated effector-like T cells and attenuate T-cell senescence, it is tempting to speculate that these parameters are of particular importance for sustaining T-cell activities during chronic infections.[58,59]

Suppressive cytokines: IL-10 and transforming growth factor-β

The composition of the cytokine milieu can have both a positive and a negative influence on the development of adaptive immune responses. Several chronic infections including Epstein–Barr virus (EBV), HBV, HCV, HIV and LCMV are associated with the production of increased levels of the immunosuppressive cytokine, IL-10.[60–67] In addition to the production of endogenous IL-10 by the infected host's own cells, EBV, cytomegalovirus, as well as the Orf *Parapoxvirus*, encode IL-10 homologues, which can also negatively regulate the immune response.[68] The analyses of polymorphisms within the IL-10 promoter provide further evidence of the influence of this cytokine on the outcomes of infection. Promoter sequences associated with low IL-10 production are more prevalent in individuals with asymptomatic HBV infection, and those associated with high IL-10 production are a risk factor for the development of chronic HCV infection.[69–71] Interleukin-10 has multiple effects and has been shown to reduce pro-inflammatory cytokine production, impede the functions of antigen-presenting cells, dampen T-cell responses and also affect B cells.[68,72] This cytokine is produced by CD4 T cells, including regulatory T cells, as well as by many other cell types such as dendritic cells, macrophages, B cells and CD8 T cells.[68,72]

The relationship between IL-10 and T-cell exhaustion has been well studied using the LCMV system. Comparative analyses of mice undergoing acute (LCMV-Armstrong) and chronic (LCMV clone 13) infections revealed that both IL-10 protein and messenger RNA levels were higher in the chronically infected cohort.[63–65] Because chronic LCMV infection is associated with T-cell exhaustion and elevated levels of IL-10 are observed under these conditions, the next steps were taken to test whether reducing the availability of IL-10 would enhance responses and promote viral control. The administration of antibodies that block the IL-10 receptor immediately following infection as well as a therapeutic regimen given after the infection had taken hold resulted in lower viral loads, decreased expression of PD-1, and improved the functionality of the virus-specific T cells.[63,64] Significantly, these changes occurred without any signs of overt immunopathology. Additional studies in which neutralizing anti-IL-10 antibodies were administered also showed comparable initial effects; however, the infection was not cleared and exhaustion was observed at later time-points highlighting the central importance of antigen persistence in sustaining exhaustion.[65]

The utility of IL-10 blockade approaches for rejuvenating exhausted responses and accelerating viral clearance has been further shown following therapeutic vaccination, again in the setting of chronic LCMV infection. Therapeutic DNA vaccination alone was ineffective; however, when given together with anti-IL-10 receptor antibodies, virus-specific CD4 and CD8 T-cell responses were enhanced and viral loads were better contained.[73] Collectively, the findings from the studies outlined above demonstrate that the levels of a single cytokine, in this case IL-10, can have a profound influence on the outcome of infection and the quality of the cellular immune response. However, it is not yet defined whether exhaustion is the result of the direct actions of IL-10 on T cells. Moreover, although results indicate that IL-10 and PD-1 promote and maintain exhaustion independently, less is known regarding how the roles of IL-10 integrate with additional parameters, including the levels of other cytokines, that determine the fate of the response.[74]

The immunosuppressive cytokine transforming growth factor-β (TGF-β) has been implicated in regulating the size of the pathogen-specific T-cell responses and the propensity of these cells to undergo apoptosis.[75,76] This is especially relevant during chronic infections as blunted initial responses are often observed and severely exhausted cells succumb to deletion. During acute infections, TGF-β restricts the size of both the effector and memory CD8 T-cell pool, most likely by inducing the expression of the pro-apoptotic protein Bim, as well as by down-regulating the expression of the anti-apoptotic gene Bcl-2.[75] The significance of the TGF-β pathway on the development of exhaustion has been further dissected following LCMV clone 13 infection. Interestingly, elevated levels of TGF-β are observed under these conditions and the TGF-β pathway appears to regulate the size of the response but not directly influence the functional capacity of the cells.[76] Nevertheless, circumventing this attenuation of the

response was beneficial because increasing the numbers of virus-specific T cells brought about viral clearance.

Whether TGF-β and IL-10 levels are universal determinants of exhaustion during all types of persistent infections remains undefined. It is plausible that other cytokines, such as IL-27 or IL-35, also influence the quality of anti-viral T-cell responses. Interleukin-27 regulates the immune response during certain parasitic infections and autoimmune diseases by dampening T-cell activities, whereas IL-35 secretion by regulatory T cells is associated with immunosuppression, making either of these cytokines excellent candidates for promoting T-cell exhaustion.[77,78]

Inhibitory receptors

As T-cell responses are elicited following infection, dramatic changes occur in gene expression, including the up-regulation of inhibitory receptors.[33,38] During acute infections these receptors function to limit the severity of the response but are then down-regulated as the pathogen is cleared and the memory pool forms. This pattern diverges during chronic infections and the establishment of the exhausted state is associated with the constitutive expression of constellations of inhibitory receptors, which collectively operate to negatively regulate the functional and proliferative potential of the responding cells. The identification of the importance of inhibitory receptors in the dysregulation of cellular immune responses in chronically infected hosts has revealed new potential therapeutic targets for restoring immune functions and decreasing viral loads.

The significance of the CD28 family member PD-1 (CD279) in exhaustion was first discovered following micro-array analysis of the gene expression profiles of virus-specific CD8 T cells during chronic LCMV infection.[79] PD-1 plays a role in establishing peripheral tolerance and inhibits the proliferation and function of T cells. In the LCMV system, PD-1 is markedly up-regulated on exhausted T cells but only transiently expressed on effector T cells during acute infections, and is not present on functionally competent memory T cells. Blocking anti-PD-L1 antibody treatment during chronic LCMV infections promotes the proliferation of virus-specific T cells, improves their functionality, and reduces viral loads, even in cohorts of CD4-depleted mice, which develop severe T-cell exhaustion.[79]

During HIV-1 infection PD-1 is expressed by both virus-specific CD8 and CD4 T cells.[80–82] Notably, the levels and frequency of PD-1 expression positively correlate with viral loads and inversely correlate with CD4 T-cell counts. The expression is also dynamic and declines following the initiation of anti-viral therapy. In long-term non-progressors the levels of PD-1 are low on HIV-specific T cells and these populations are more polyfunctional. *In vitro* studies show that blocking the PD-1 pathway allows exhaustion to be overcome by enhancing the proliferation of CD8 and CD4 T cells and promoting cytokine production.[80–82] Significantly, blocking PD-1 antibody treatments during the early or later phases of simian immunodeficiency virus (SIV) infection showed remarkably promising results. The frequencies and functional quality of SIV-specific CD8 T cells detectable in the blood and gut increased, viral loads dropped, and the survival rate of the infected macaques improved.[43] The dynamics and significance of PD-1 expression has also been examined following HBV and HCV infections.[3,22,24,83,84] PD-1 levels are up-regulated on virus-specific CD8 T cells during the acute phase of the infection, but tend to be lower if the infection is resolved; however, higher PD-1 expression is associated with an exhausted phenotype in individuals with chronic infections.[3,22,24,83,84] PD-1 expression is markedly up-regulated on HCV-specific CD8 T cells in the liver; however, the function of these cells is not enhanced by PD-1 blockade.[84] This illustrates that multiple factors must regulate the maintenance of exhaustion and also suggests that the severity of exhaustion is impacted by localized levels of viral antigen and the compartmentalization of the T cells.

Although PD-1 is the best characterized inhibitory receptor associated with exhaustion, several other receptors have also been shown to impair T-cell responses during chronic infections. Cytotoxic T-lymphocyte antigen 4 (CTLA-4), which like PD-1 is a CD28 family member, has been shown to impact the functional quality of the T-cell response during HIV-1 and HCV infections of humans.[85–87] Treatment of SIV-infected macaques with blocking CTLA-4 antibodies reveals some of the potential complications of 'inhibiting inhibitors' to promote immune responses and viral control. Viral loads were higher in infected macaques administered CTLA-4 blocking antibodies from the onset of infection, and treatment at later time-points reduced the effectiveness of anti-retroviral chemotherapy.[86] These changes may be attributable to increased T-cell activation caused by releasing the cells from the attenuating effects of CTLA-4 and consequently providing a greater pool of infectable and infected targets.

The importance of counterbalancing potentially detrimental responses with the ability to elicit and sustain sufficiently protective T-cell responses is further exemplified by studies of T-cell immunoglobulin and mucin domain-containing protein-3 (TIM-3), because this receptor functions to attenuate autoimmune responses and has also been shown to influence the exhausted state. During HIV-1, HCV and LCMV infections TIM-3 is expressed by virus-specific T cells, and the frequencies and levels of expression parallel the exhausted state of the cells and the severity of infection.[8,88,89] As with PD-1, blockade of TIM-3 improves the responsiveness and proliferation of the exhausted cells *in vitro*, but interestingly, TIM-3 is not necessarily co-expressed with PD-1 and can therefore identify a distinct subset of exhausted but PD-1-negative T cells.[8,88]

The hierarchies of inhibitory receptor expression by exhausted cells have been documented during chronic LCMV infection, and these populations of CD8 T cells can be segregated into a series of discrete subsets that express different numbers and combinations of inhibitory receptors.[33,38] More severely exhausted cells express a greater number of inhibitory receptors. The roles of each individual receptor in promoting and sustaining exhaustion are less clear. The inhibitory molecule lymphocyte-activated gene-3 (LAG-3) is widely expressed on exhausted LCMV-specific CD8 T cells.[38,89] Nevertheless, LAG-3 blockade alone is less effective at reversing exhaustion and lowering viral levels than a combined PD-L1 and LAG-3 blocking approach; moreover, the establishment of exhaustion occurs similarly in wild-type and LAG-3-deficient mice.[38,89] Therefore, deciphering how specific inhibitory receptor signals integrate to promote and maintain exhaustion will be important next steps.

Molecular control of exhaustion

Exhausted T cells can be distinguished from typical effector and memory cells in many ways (Fig. 1).[33] As the exhausted state develops in a stepwise, progressive, and often predictable manner, it is plausible that distinct, but overlapping, mechanisms independently regulate successive waves of changes in cellular activities such as susceptibility to apoptosis, proliferative capacity, ability to elaborate select effector functions, and receptor and adhesion molecule expression. This modular regulatory process is supported by studies of exhausted LCMV and HIV-specific T cells showing that defective nuclear translocation of nuclear factor of activated T cell 2 (NFAT-2) ablates IFN-γ secretion while degranulation and killing functions remain intact.[30]

Cbl-b is a RING-type E3 ubiquitin ligase that down-modulates TCR signalling and is strongly induced in anergic T cells.[90] Deletion of Cbl-b results in independence from co-stimulation, hyperproliferation and enhanced IL-2 production. During acute LCMV infection Cbl-b deficiency prevents TCR down-regulation and increases the levels of IFN-γ production but does not impact cytotoxicity.[91] During chronic LCMV infections the loss of Cbl-b restores IFN-γ production and confers resistance to deletion.[92] This enhancement of the response increases morbidity and mortality, exemplifying the importance of exhaustion in preventing immunopathology. Gene related to anergy in lymphocytes (GRAIL) is also an E3 ubiquitin ligase that is associated with anergy; however, this molecule is not markedly up-regulated in exhausted LCMV-specific CD8 T cells.[33,93] This further supports the concept that a unique ensemble of molecular regulators governs the exhausted setting and distinguishes these cells from conventional anergic, effector and memory T cells.

The transcriptional repressor BLIMP-1 (Pdrm1) regulates the terminal differentiation of CD8 T cells and is highly expressed during chronic infections.[94] In the LCMV system, BLIMP-1 deficiency in T cells prevents the development of some of the characteristic signatures of exhaustion as the cells express CD127, retain the ability to produce IFN-γ and IL-2, and express fewer inhibitory receptors despite a failure to contain the infection.[94] Intriguingly, haplo-insufficiency of BLIMP-1 enhances immune-mediated viral control. The levels rather than the presence or absence of BLIMP-1 critically influences the outcome of the infection and quality of the T-cell response so it will be important to fully understand how BLIMP-1 expression is regulated. One regulator of BLIMP-1 is IL-21, as IL-21 signalling can induce BLIMP-1 in pre-activated CD8 T cells; however, additional factors must regulate BLIMP-1 expression because this molecule is required for exhaustion, which is augmented by the absence of IL-21.[49–51,95]

Future prospects

In many ways the factors that promote and maintain exhaustion are still being defined. As more regulatory parameters are uncovered, it will be necessary to dissect how these individual mechanisms network together to fine-tune the phenotypic and functional set-points of the response. Gene-expression analyses suggest that metabolic defects manifest in exhausted T cells, and several recent reports have implicated changes in cellular metabolism and the mammalian target of rapamycin pathway in the generation of effector and memory T-cell responses.[33,96–98] It will be of interest to determine whether and how these pathways influence the quality of T-cell responses during chronic infections. Importantly, several approaches have now been shown to improve responses and in certain cases lower viral loads. Nevertheless, whether all subsets of exhausted cells are susceptible to these regenerative methods, and whether these cells are fully reactivated and truly comparable in function and proliferative potential to bona fide memory cells, requires further evaluation. The key will be to devise tailored approaches, possibly using a combination of strategies that promote viral clearance while avoiding immunopathology and unintended consequences such as enhanced infection, autoimmunity or a deterioration of responses.

Disclosure

The authors have no conflicts of interests to disclose.

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